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(71) Applicant: **Dr. Ruhland Nachf. GmbH, Stadtplatz 7, D-8425 Neustadt, Danube (Germany)**

(72) Inventor: **Dr. Axel Sternberger, Cramer Klett Strasse 35e, D-8014 Neubiberg (Germany)**

(74) Representative: **Dr. Bernd Hansen et al., Hoffmann, Eitle & Partners Patent Attorneys, Arabellastrasse 4, D-8000 Munich 81 (Germany)**

(54) **Tissue Bondable Collagen Wound Dressing**

A flat, tissue bondable collagen wound dressing consisting of collagen and fibrinogen in a layered structure containing a collagen layer 0.3 to 2 cm thick is described; it has a fibrinogen layer 0.2 to 2 mm thick on at least one surface and contains fibrinogen in the amount of 0.5 to 10 mg/cm^2 and permits improved wound healing.

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Tissue Bondable Collagen Wound Dressing

Collagen has been used in surgery for a long time. It can be used in the form of sponges or fibers for hemostasis; after appropriate modification, it is also suitable for accelerating wound healing. In patients with a coagulation disorder or in cases of bleeding over a large area, the usual collagen wound dressings are not sufficient. Therefore, there have already been attempts to bond collagen material to tissue by using adhesives based on resorcinol-formaldehyde, collagen or gelatin. Although such adhesives are hemostyptic, they are not suitable for practical use because of their toxicity. This is also true of acrylate adhesives and their combination with collagen wound dressings.

It is known that collagen can enter into covalent crosslinking bonds with components of connective tissue. Crosslinking with collagen takes place by way of Schiff bases and aldol condensation. It is also known that in the case of basal membranes, tissue strength is also achieved by means of S-S bridges of the basal membrane collagen. It is also known that proteins such as albumin can be crosslinked with intermolecular S-S linkages after mild reduction and subsequent oxidation over S-S bridges.

In the case of injuries, primary wound closure occurs due to coagulation of blood. Aggregated platelets and a fibrin network are responsible for this effect. It is known that individual fibrin molecules are crosslinked by transglutaminases (such as factor XIII). New peptide linkages are then formed between the glutamic acid and lysine of adjacent chains. The final phase of plasmatic coagulation of blood is simulated by the technique of fibrin bonding, i.e., the use of fibrinogen and thrombin.

The fibrin bonding alone is not capable of stopping bleeding over a large area. This is possible only through combined use of fibrin bonding with an absorbable collagen wound dressing. However, one must then keep three components on hand for this operation, namely the collagen wound dressing, the thrombin solution containing antifibrinolytics and a deep-frozen highly concentrated fibrinogen solution. Since the occurrence of bleeding is often sudden and unscheduled, these three components are not always available and ready to use at the crucial moment, because the deep-frozen fibrinogen solution at least must be thawed out first before proceeding. Furthermore, the procedure of mixing the components before application is relatively complicated.

In the journal *Wiener medizinische Wochenschrift* [Viennese Medical Weekly Journal], No. 7 (1976) pages 86 through 89, local use of fibrinogen and collagen for hemostasis in heart surgery is reported. Human fibrinogen cryoprecipitate, thrombin, a factor XIII concentrate and a collagen nonwoven are used in the bonding technique described there. Fibrinogen is applied to the desired location and is coagulated there by adding thrombin solution and factor XIII. In the case of severe bleeding, the collagen nonwoven is used as a completely absorbable carrier substance, so that the solutions are applied to the collagen nonwoven and then the collagen is pressed onto the bleeding wound with the fibrinogen coated side facing the wound. This should result in complete polymerization of the fibrin and bonding of the fibrin fibers in the tissue. In this bonding technique, the collagen is only coated with fibrinogen immediately before use in order to mostly prevent the collagen sponge from collapsing.

The object of this invention is to make available to surgeons a collagen wound dressing which can be used in a prepared form directly during surgery and in which the above-mentioned disadvantages of fibrin adhesion in combination with absorbable collagen do not occur. In conjunction with this object, another goal is to make available a collagen preparation that is suitable for storage and contains all the components needed to induce local hemostasis.

This object is achieved by a tissue bondable collagen wound dressing according to Claim 1.

The wound dressing according to this invention has a layered structure. The main layer here consists of collagen, and this layer has a thickness between about 0.3 and 2 cm, preferably 0.7 to 1 cm. A fibrinogen layer is applied to one or both sides of this collagen layer. This fibrinogen layer has a thickness of about 0.2 to 2 mm and is applied to the collagen surface, with the fibrinogen molecules being anchored to the collagen molecules in the borderline area of the layers.

It is essential that the fibrinogen layer contains fibrinogen in an amount of 0.5 to 10 mg/cm^2, preferably 4 mg/cm^2.

It is often advantageous if the fibrinogen, which is applied as a layer to one or both sides of the collagen, contains free SH groups. These free SH groups may first be formed in the fibrinogen by reductive cleavage of disulfide bridges or by mixing fibrinogen with fibrinogen which contains SH groups. In addition, it is also possible to leave the disulfide bridges in the fibrinogen unchanged and to incorporate additional SH groups, e.g., according to the method of Benesch and Benesch as reported by Stephen et al. in *Proceedings of the National Academy of the USA, Washington*, vol. 44 (1958) pages 848 to 853 or *Biochem. Journal* vol. 101 (1966) pages 717 to 720.

It may be advantageous if, in addition to the fibrinogen, at least one layer also contains antifibrinolytics and/or polyvalent proteinase inhibitors, in which case thrombin may also be present, with the provision that the thrombin is not present in the same layer or in direct contact with the fibrinogen.

The collagen wound dressing according to this invention can be produced by the following method:

First, collagen is prepared in a known manner. The collagen should preferably have a purity, expressed by the weight ratio of nitrogen to hydroxyproline, of <4, in particular <3:

$$\text{factor N/hyp} = (\text{mg N})/(\text{mg 4-hyp})$$

J. Mol. Bio. vol. 44 (1969) page 161.

A 1.5% collagen solution and 0.05% acetic acid is freeze dried in a known way to form a 0.5 cm thick collagen sponge. If desired, a solution to which antifibrinolytics (tranexamic acid) and/or polyvalent proteinase inhibitors (aprotinin) have previously been added may also be used in freeze drying, in which case these substances are added in amounts such that the concentration in the finished collagen sponge is 0.2 to 10 mg/cm^2 or 4 to 1000 KIU.

A fibrinogen solution is prepared as follows, separately from the preparation of the collagen sponge:

Fibrinogen for infusion purposes is dissolved in isotonic NaCl up to a concentration of 30 mg fibrinogen per mL. The solution is then sprayed using a conventional sprayer device onto the previously prepared collagen sponge under sterile conditions in a thickness of 0.2 to 2 mm, corresponding to an amount of 0.5 to 10 mg/cm^2, then freeze-dried immediately, in which case the resulting layer thickness usually must not exceed 2 mm, because otherwise adhesion of this layer is poor. Using this technique, adequate anchoring in the collagen matrix is ensured. If desired, antifibrinolytics and/or polyvalent proteinase inhibitors may also be incorporated into the fibrinogen layer.

It is known that collagen may be used as a carrier for antibiotics such as gentamicin. The wound dressings according to this invention may also contain active ingredients such as gentamicin, tetracycline or other antibiotics or chemotherapeutics.

Preparation of Collagen :

Fresh bovine tendon freed of all pigment layers and muscle residues was homogenized, and an amount corresponding to 100 g dry weight was extracted in three liters of 0.05 M citrate buffer (pH 3.7) for 24 hours and then dialyzed against 1% acetic acid for 12 hours.

Tissue suspended in three liters of 1% acetic acid was incubated with pepsin in a collagen: pepsin ratio of 50:1 for 48 hours at 10°C while stirring constantly.

The batch was diluted to five liters with 1% acetic acid and freed of undissolved tendon fragments by centrifugation.

The viscous collagen solution was dialyzed against alkalized tap water (pH 8.0) and then centrifuged sharply. The residue was again dissolved in five liters of 1% acetic acid and dialyzed. This procedure was repeated until the ratio of nitrogen to 4-hydroxyproline was <3. After the last dialysis, a 1.5% collagen solution was prepared with 0.05% acetic acid and then used for the following experiments.

To prepare collagen sponge with the dimensions 10 x 10 x 0.5 cm, containing antifibrinolytics and/or proteinase inhibitors, 50 mL of the collagen solution was mixed with 0.2 g tranexamic acid and/or 40,000 KIU aprotinin and then freeze-dried.

To prepare a collagen-gentamicin sponge of 10 x 10 x 0.5 cm, 50 mL of the collagen solution was adjusted to a pH of 1 to 2, mixed with 100 mg gentamicin sulfate and then freeze-dried.

Preparation of Fibrinogen Solution:

Commercially available sterile fibrinogen was dissolved in sterile isotonic Na HCl [sic; NaCl?] to yield a solution of 30 mg fibrinogen per mL solution, which was used for the following experiments.

To obtain fibrinogen modified with SH groups, the procedure is as follows:

Dissolve fibrinogen in isotonic saline solution up to a concentration of 20 mg/mL. Mix 10 mL of this fibrinogen solution with 1 mL of an N-acetylhomocysteine thiolactone solution (60 mg/mL distilled water) and 10 mL of a carbonate buffer (pH 10.6) and incubate for 35 minutes at 0°C. Then stop the reaction by adding 40 mL of a phosphate buffer, pH 7.0 i 0.4 [sic; 7.0 ± 0.4 ?]. Then deionize the solution and concentrate it at the same time by the membrane filtration technique using a filter (molecular weight exclusion limit 5,000).

<u>Preparation of Wound Dressings containing Collagen and Fibrinogen</u>:

First, add 100 mL of a 1% collagen solution to a metal mold measuring 10 x 10 cm, then freeze dry according to the usual technique and sterilize the resulting sponge. Working under aseptic conditions, spray this sterilized collagen sponge with a fibrinogen solution in an amount so that 5 mg fibrinogen is applied per square centimeter of collagen surface. Then freeze dry again and package under sterile conditions. The collagen layer is 10 mm thick and the fibrinogen layer on top of it is approximately 0.3 mm thick.

Results of the *in vitro* and *in vivo* experiments with collagen-fibrinogen sponges and gentamicin-collagen-fibrinogen sponges.

<u>In vitro</u>:

Wound dressings prepared in this way were tested with a tensile strength testing machine consisting of a force pickup connected to a recorder. The machine was calibrated in the range of 100 to 1000 grams of force. The collagen-fibrinogen sponges were glued to a plastic disk (diameter = 1 cm) on the collagen side by using a commercial adhesive. The side coated with fibrinogen was then moistened with 0.1 mL thrombin solution (1000 NIH/mL) and the tensile strength was determined after 20 minutes in the test machine. This yielded values of 730 g/cm^2 (average of seven measurements).

<u>In vivo</u>:

In a study on 400 rats, the (gentamicin) collagen-fibrinogen wound dressings (known as Fibrocoll) were tested for securing an anastomosis on the colon with the traditional tissue adhesive with plasma fractions (fibrin bonding system = FK).

a) Control group (enterotomies with seven inverting single button sutures according to Lembert)

b) Additional application of Fibrin bonding system

c) Fibrin bonding system with collagen nonwoven (free of fibrinogen and gentamicin)

d) Securing sutures with Fibrocoll

e) Fibrocoll with gentamicin (1 mg/cm^2)

In the initial phase of wound healing, the animals treated with the different adhesive systems showed a much higher wound strength in the suture area up to the third day postoperatively. In comparison with the traditional fibrin adhesive technique, adhesions in the wound area were reduced by using Fibrocoll. Due to the application of the gentamicin-collagen-fibrinogen wound dressings, controlled release of the antibiotic was observed and serum concentrations were far below the toxic limit.

In another series of experiments, hemihepatectomies were performed in the "finger fracture technique" on five pigs, and after ligature of the arteries and veins that could be picked up, the bleeding parenchyma defects were treated with collagen-fibrinogen wound dressings. No secondary bleeding was ever observed in the following postoperative course, and absorption of the materials was concluded after 12 weeks.

With these (gentamicin) collagen-fibrinogen wound dressings, a material is now available which is simple, reliable and quick to use and is excellent for hemostasis. In addition, an improvement in wound healing is observed together with concomitant antibiotic protection.

Patent Claims:

1. A dry collagen wound dressing in the form of a nonwoven or sponge that can be bonded to tissue and consists of collagen and fibrinogen, characterized in that it is composed of layers consisting of a combination of collagen and fibrinogen by freeze drying and it contains a collagen layer 0.3 to 2 cm thick, having a fibrinogen layer 0.2 to 2 mm thick on at least one surface containing fibrinogen in the amount of 0.5 to 10 mg/cm^2 anchored in the collagen.

2. A wound dressing according to Claim 1, characterized in that the fibrinogen contains SH groups.

3. A wound dressing according to Claim 2, characterized in that the fibrinogen contains SH groups which are either introduced into the fibrinogen molecule by subsequent sulfhydration or are formed by reducing the disulfide bridges in the fibrinogen molecule.

4. A wound dressing according to one of the preceding claims, characterized in that it also contains an active drug ingredient.

5. A wound dressing according to Claim 4, characterized in that it contains antifibrinolytics and/or polyvalent proteinase inhibitors and/or thrombin in at least one of the layers, but fibrinogen and thrombin are not combined in one layer.

6. A wound dressing according to Claim 4, characterized in that the active drug ingredient is an antibiotic.

7. A wound dressing according to Claim 6, characterized in that the antibiotic is gentamicin.

8. A wound dressing according to Claim 1, characterized in that the collagen has a purity of <4, preferably <3, expressed by the weight ratio of nitrogen to 4-hydroxyproline.